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ORIGINAL ARTICLE

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Investigation of oxidative damage, antioxidant balance, DNA repair genes, and apoptosis due to radiofrequency-induced adaptive response in mice

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ABSTRACT

This study aims to determine whether exposure to non-ionizing radiofrequency fields could induce an adaptive response (AR) in adult mice and to reveal potential molecular mechanisms triggered by RF-induced AR. The study was performed on 24 adult male Swiss-Albino mice. The average mass of the mice was 37 g. Four groups of adult mice, each consisting of 6, were formed. The radiofrequency group (R) and the adaptive response group (RB) were exposed to 900 MHz of global system for mobile communications (GSM) signal at 0.339 W/kg (1 g average specific absorption rate) 4 h/day for 7 days, while the control group (C) and the bleomycin group (B) were not exposed. 20 minutes after the last radiofrequency field (RF) exposure, the mice in the B and RB groups were injected intraperitoneal (ip) bleomycin (BLM), 37.5 mg/kg. All the animals were sacrificed 30 minutes after the BLM injection. Oxidative damage and antioxidant mechanism were subsequently investigated in the blood samples. Changes in the expression of the genes involved in DNA repair were detected in the liver tissue. TUNEL method was used to determine the apoptosis developed by DNA fragmentation in the liver tissue. The RB group, which produced an adaptive response, was compared with the control group. According to the results, the increase of reactive oxygen species (ROS) in the RB group may have played an important role in triggering the adaptive response and producing the required minimum stress level. Furthermore, tumor suppressor 53(p53), oxo guanine DNA glycosylase (OGG-1) levels responsible for DNA repair mechanism genes expression were increased in conjunction with the increase in ROS. The change in the poly (ADP-ribose) polymerase 1 (PARP-1) and glutathione peroxidase 1 (GPx-1) gene expression were not statistically significant. The antioxidant enzyme levels of superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) were decreased in the group with adaptive response. According to the data obtained from terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis, apoptosis was decreased in the RB group due to the decrease in cell death, which might have resulted from an increase in gene expression responsible for DNA repair mechanisms. The results of our study show that exposure to RF radiation may create a protective reaction against the bleomycin. The minimal oxidative stress due to the RF exposure leads to an adaptive response in the genes that play a role in the DNA repair mechanism and enzymes, enabling the survival of the cell.

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KEYWORDS
Radiofrequency radiation; adaptive response; stress response; DNA oxidative damage; bleomycin

Introduction

Radiofrequency fields classified as non-ionizing radiation in the range from 30 kHz to 300 GHz band of the electromagnetic spectrum are used not only in medicine, but also in the technologies such as mobile phones and wireless internet, which are indispensable parts of our daily lives. Since the International Commission of Non Ionizing Radiation Protection (ICNIRP) released recommendations for human exposure in 1998, the relationship between radiofrequency electromagnetic fields (EMFs) and adverse health effects have been further examined by a substantial community of science groups (ICNIRP 2020). International Agency for Research on Cancer (IARC) has classified radiofrequency electromagnetic fields (RF-EMF) as potentially carcinogenic to humans (IARC 2017). Considering the potential effects of RF-EMF exposure, usage of wireless devices has become a sensitive matter of balance between public health and social gains. There are also several investigations on the favorable and possible harmful effects of RF-EMF exposure (Bernhardt 1992; Gherardini et al. 2014).

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Bleomycin is an antineoplastic chemotherapy agent causing chromosomal aberrations, so it has been used in therapeutic chemotherapy to treat different cancers (Bolzán and Bianchi 2018; Schlade-Bartusiak et al. 2002; Zong et al. 2015). The exposure of cells or animals to low dose non-ionizing radiation, ionizing radiation, or chemical agent, to subsequently become resistant to toxic damage caused by exposure to a challenge dose (CD) of ionizing radiation or chemical agent is called adaptive response (Vijayalaxmi and Scarfi 2014. The adaptive response phenomenon was first demonstrated in Escherichia coli in 1977 (Samson and Cairns 1977). The non-toxic dose of mutagens is usually referred to as adaptation dose (AD) and the toxic dose of mutagens as a CD. The challenge dose of mutagens causes significant damage to cells and organisms. Studies exhibited that this damage was reduced with the adaptive response effect when RF was used before the CD (Falone et al. 2018; Zong et al. 2015). For example, the latest experiments have showed that the exposure to RF before CD of chemical agents (bleomycin, etc.) (Sannino et al. 2011; Zeni et al. 2012; Zong et al. 2015) or ionizing radiation (Cao et al. 2011; Jiang et al. 2013) in in-vivo and in-vitro conditions dramatically reduced the levels of DNA damage when compared to CD only.

Along with the adaptive response caused by oxidative stress, some genes play an important role in inducing the repair mechanism of the cells. Tumor suppressor 53 (*p53)* plays a role in maintaining genomic integrity and eliminating damaged DNA (Bargonetti and Manfredi 2002). However, its role in the adaptive response induced by RF has not been determined. In recent in-vitro studies, the exposure of RF-EMF before challenge dose increases poly (ADP-ribose) polymerase 1 (*PARP-1)* gene expression in various cell types (He et al. 2016, 2017), and the findings show that *PARP-1* plays a role in AR caused by pre-exposure RF-EMF. Another gene involved in the DNA repair mechanism is oxo guanine DNA glycosylase (*OGG-1)* (Sampath et al. 2012). In a study conducted in 2018, the expression level of these genes decreased in the group treated with CD, but the group treated with pre-exposure RF also demonstrated similarity with the control group in in-vitro condition (Falone et al. 2018).

While reactive oxygen species (ROS) play a role in oxidative stress, malondialdehyde (MDA) play a role in lipid oxidative damage and catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity (TAC) play a role in antioxidant mechanisms. The formation of excessive ROS can lead to cell damage by causing oxidative stress, resulting in cell death. Cells have antioxidant mechanisms to balance the ROS increase. ROS increase and its inactivation by antioxidant mechanisms lead to homeostasis, but the increase of free radicals disrupts this balance and causes cell damage (Poljsak et al. 2013). Enzymes such as glutathione peroxidase (GPx), SOD, and CAT help maintain redox balance within the cell to prevent the harmful effects of oxidative stress (Zuo et al. 2015).

There are many studies on the effect of RF-EMF on DNA damage and free radical metabolism (Akdag et al. 2016; Alkis et al. 2019; Dasdag et al. 2009). There are few studies in the literature regarding the development of AR against the mutagen agent by inducing RF in in-vivo conditions. Researchers have evaluated the development of AR against oxidative stress with the change in the expression of different genes, and the activation of many signal mechanisms. More effective detoxification of free radicals and the enhancement of antioxidant mechanisms and the activation of DNA repair systems are thought to play a role in the development of AR (Dimova et al. 2008; Vijayalaxmi and Scarfi 2014). In this research, our aim is to investigate the RF-EMF induced AR against oxidative mutagens in in-vivo experiments, evaluate them as a whole with the mechanisms in the genes and protein levels, and apoptosis.

In this study, mice were exposed to RF-EMF (900 MHz pulsed, mobile phone signal) in a distinct group and subsequently treated with BLM (37.5 mg/kg). Potential molecular mechanisms of RF-EMF induced RB group were compared with the control group. To evaluate oxidative damage (ROS) and lipid oxidative damage (MDA), antioxidant mechanism; CAT, SOD, and TAC were analyzed by the ELISA method in the blood samples. The changes in the expression of genes involved in DNA repair *p53, PARP-1, OGG-1*, and *GPx-1* were analyzed with the qRT-PCR method in liver tissues taken from the experimental groups. TUNEL method was used to determine apoptosis developed by DNA damage in the liver tissue.

Methods

Materials

BLM 37.5 mg/kg (Nippon Kayaku Co. Ltd, Tokyo, Japan), Ethylene-diamine-tetra-acetic acid (EDTA) (Ayset Co., Adana, Turkey), Catalase Activity Colorimetric/Fluorometric Assay Kit (BioVision, Cat: K773), Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (BioVision, Cat: K739), Reactive Oxygen Species (ROS) Detection Assay Kit (BioVision, Cat: K936), Superoxide Dismutase (SOD) Activity Assay Kit (BioVision, Cat: K335), Total Antioxidant Capacity (TAC) Colorimetric Assay Kit (BioVision, Cat: K274), PureZOL RNA isolation reagent

(Bio-Rad, Cat: 7326890), Water DEPC (0.1%) (Serva, Cat: 39798.03), the cDNA were used for the SsoFast™ EvaGreen® Supermix (Bio-Rad, Cat: 1725201), and primers were synthesized by Oligomer (Ankara, Turkey), ApopTag Peroxidase in Situ Apoptosis Detection kit (ApopTag Peroxidase in SituApoptosis Detection kit, Cat: S7101, Millipore, Billerica, MA).

Animal procurement and handling

The Experimental Animals Ethics Committee of Gazi University approved the experimental protocol (approval number: G.Ü. ET-19.038). This research was conducted in the Laboratory of Animals Breeding and Experimental Research Center of Gazi University in line with experimental ethical standards and animal care laws, according to the rules and regulations in Turkey. All the animals were kept in a room with controlled temperature of 22 degrees celsius (°C), humidity 50 ± 5%, and 14/16 h light/dark cycle conditions in plexiglass cages. The mice were sacrificed by cardiac puncture following anesthesia with intraperitoneal injection of ketamine (200 mg/kg) and xylazine (10 mg/kg).

RF exposure conditions

In the experimental setup, a vector signal generator (SMBV 100A, 9 kHz to 3.2 GHz; Rohde & Schwarz, Munich, Germany) was used to produce the RF radiation. The generator produced a 900 MHz GSM signal as described in the 3rd Generation Partnership Project (3GPP) standard with an average signal power of 250 mW. The electric field of the applied RF field was determined by electric field probe (EMR 300, Narda, Germany). The RF signal from the generator was applied to a wideband horn antenna (ETS Lindgren, 3164–04, USA). A plastic cage of polymethyl methacrylate (15 x 23 × 33 cm) was used to house the mice. When the signal generator was turned on, the electric field was measured as 20–21 V/m. When the generator was turned off, the background electric field was measured as 0.1–0.20 V/m.

Experimental design

The study was performed on 24 adult male Swiss-Albino mice. The mass of the animals was measured during the experiment and there was no statistically significant difference between the groups. The average mass of the mice was 37 g. The mice were randomly divided into 4 equal groups as shown in Table 1.

Table 1. Experimental groups.

Groups	n	RF-EMF Exposure	BLM
Control (C)	6	Inactive exposure	Without injection
RF exposure (R)	6	RF exposure	Without injection
Bleomycin (B)	6	Without exposure	İntra-peritoneal injection
RF+Bleomycin (RB)	6	RF exposure	İntra-peritoneal injection

Group C: *Sham (no exposure/no injection)*: Daily for seven days, they were then kept individually in a cage for 4 h/day between 9:30 AM and 01:30 PM without exposure to RF-EMF. These animals served as sham exposed injection-controls.

Group R: *RF-exposure/no injection*: Animals exposed to 900 MHz RFR for 4 h/day between 9:30 AM and 01:30 PM for 7 days.

Group B: *no exposure/BLM injection*: Daily for seven days, they were then kept individually in a cage for 4 h/day between 9:30 AM and 01:30 PM without exposure to RF-EMF. BLM (37.5 mg/kg ip) was injected intraperitoneally 20 minutes later of RF-EMF exposure in the last day of the experiment.

Group RB: *RF-exposure/BLM injection*: They exposed to 900 MHz RF-EMF for 4 h/day between 9:30 AM and 01:30 PM for 7 days and BLM (37.5 mg/kg ip) was injected intraperitoneally 20 minutes later of RF-EMF exposure in the last day of the experiment.

Vijayalaxmi and Scarfi (2014) indicated that AR was not elicited instantaneously but required certain time interval between AD and CD to become fully active (Vijayalaxmi and Scarfi 2014). In our research, we adjusted the interval time according to Zong et al. (2015) (Zong et al. 2015). After the last RF-EMF exposure, the mice were kept for 20 minutes, and then the RB group was injected BLM 37.5 mg/kg i.p. 30 minutes after BLM injection, all the animals were sacrificed by cardiac puncture under general anesthesia. Bleomycin can be absorbed after subcutaneous injection (s.c.), intramuscular (i.m.), i.p., and oral administration. In mice and rabbits, the highest serum levels occur 30 minutes after a s.c. injection (Crooke and Bradner 1976; Lazo et al. 1990). We presented the research flowchart and stages in Figure 1.

Biochemical assay (ELISA)

We centrifuged blood samples at 3000 rpm for 10 minutes, and we took their plasma to eppendorf. The levels of antioxidants and oxidative damage were determined according to the plasma ROS, CAT, TAC, MDA, and SOD levels. All antioxidant and oxidative damage tests were employed in line with the standard procedures. The ROS, CAT, TAC, MDA, and SOD levels in the blood samples were determined following the

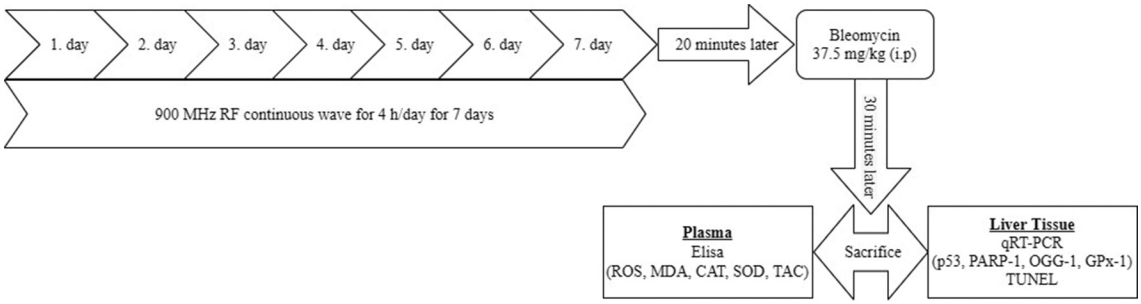


Figure 1. Experimental protocol: The heart puncture method is used to take blood samples from all mice under anesthesia. The blood sample was taken into tubes containing ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant. After the intracardiac sacrifice of the animals, the liver tissue was removed and placed in 2 ml eppendorf then put in liquid nitrogen. Samples were kept at −80°C until it was used. To evaluate the oxidative damage (ROS and MDA) and antioxidant mechanism (CAT, SOD, and TAC), blood samples were analyzed by the ELISA method. Expression levels of p53, PARP-1, OGG-1, and GPx-1 genes were analyzed by the qRT-PCR method in liver tissues taken from the experimental groups. TUNEL method was used to determine the apoptosis developed in the liver tissue.

instructions in the commercially available ELISA kit. The absorbance level was measured at Ex/Em = 495/529, 570, 570, 532, and 450 nm respectively with a microplate spectrophotometer. The ROS, CAT, MDA, SOD, and TAC levels in the plasma samples were shown in RFU 495/529, mU/mL, nmol/ml, inhibition rate %, and nmol/ml, respectively.

qRT-PCR

We isolated the total RNA from liver tissue with PureZOL RNA isolation reagent following the manufacturer's protocol. Spectrophotometry (NanoDrop ND-1000, Thermofisher) method was used to analyze the quantity and purity of harvested total RNA samples. The integrity of RNA samples was assessed by agarose gel electrophoresis under standard conditions. The obtained RNA (1000 ng) was used to synthesize cDNA by using cDNA Synthesis Kit and Bio-Rad T100 Thermal Cycler. The primers for *GPx-1, p53, PARP-1, OGG-1*, and Peptidylprolyl Isomerase A (*PPIA*) genes were designed. Working temperature of the primers was optimized before starting the gene expression analysis. Roche LightCycler 480 Real-Time PCR was used in the gene expression analysis. The list of used primers and their working temperatures are shown in Table 2. To determine the correct and reliable gene expression of the target genes in the liver samples, it is important to determine the stable housekeeping genes that are expressed uniformly in the liver tissue. Researches show that the expression of *PPIA* housekeeping genes in the liver is more stable than the other housekeeping genes (Tatsumi et al. 2008). Accordingly, *PPIA* was selected as the housekeeping gene to be used in this research for gene expression analysis.

TUNEL method

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) method was employed to identify apoptosis as a result of the DNA fragmentation in liver tissue samples of the experimental groups. ApopTag Peroxidase in Situ Apoptosis Detection kit was used to determine TUNEL-positive cells. Counterstaining was performed by Haematoxylin. The slides were evaluated under a light microscope using a computer-supported imaging system, and the pictures were taken with a light microscope (LeicaDM4000, Germany) with Leica Las V 9 program. We counted TUNEL positive cells in 4 microscopic fields at 400x magnification (Volkmann et al. 2007).

SAR

The specific absorption rate (SAR) distribution was calculated by using Computer Simulation Technology (CST AG, Darmstadt, Germany), a 3-dimensional electromagnetic field solver coupled with a thermal solver. To replicate the exposure setup, firstly, dual-ridged wideband horn antenna was modeled and verified. Then, a mouse voxel

Table 2. The list of primer.

Gene	Gene Code	Forward Primer	Reverse Primer	Temp (^{0}C)
GPx-1	NM_001329527.1	TATGTGTGCTGCTCGGCTC	TCTTCATTCTTGCCATTCTGGTG	60
p53	NM_001127233.1	TGCTCACCCTGGCTAAAGTT	AATGTCTCCTGGCTCAGAGG	59
PARP-1	NM_007415.3	CCTCTCTGTCACCTCAGCAC	ACTCATCTTCTCCACCTCCTTTTT	59
OGG-1	NM_010957.4	CCCAGAAATTCCAAGGTGTGAGA	TTGGCACTGGCACGTACATA	60
PPIA[a]	NM_008907.2	CCAAGACTGAATGGCTGGATG	AATGCCCGCAAGTCAAAAGAA	59

[a]Housekeeping gene.

model based on computerized tomography was used. Six mice under the wideband horn were simulated for electric field distribution to corroborate measured electric field values within and outside the plastic enclosure where the mice were housed. Whole body SARs were calculated in average for 1 g of tissue as 0.339 W/kg and for 10 g tissue as 0.269 W/kg using IEEE/IEC 62704–1 method. Maximum SAR in the blood and liver were observed as 0.301 W/kg and 0.255 W/kg for 1 g of tissue and 10 g of tissue in average, respectively. Mouse voxel model and SAR distributions for coronal cuts are given in Figure 2.

Statistical analysis

GraphPad Prism version 8.0.2 was used in the statistical analysis of the results. All the data are presented as means ± SEM. Data normality test was done by Shapiro-Wilk and Kolmogorov-Smirnov tests. Parametric analyses were performed by applying One-way ANOVA and multiple comparison test. P-value less than 0.05, 0.001, 0.0001 (* $p < .05$; ** $p < .001$; *** $p < .0001$) was considered statistically significant.

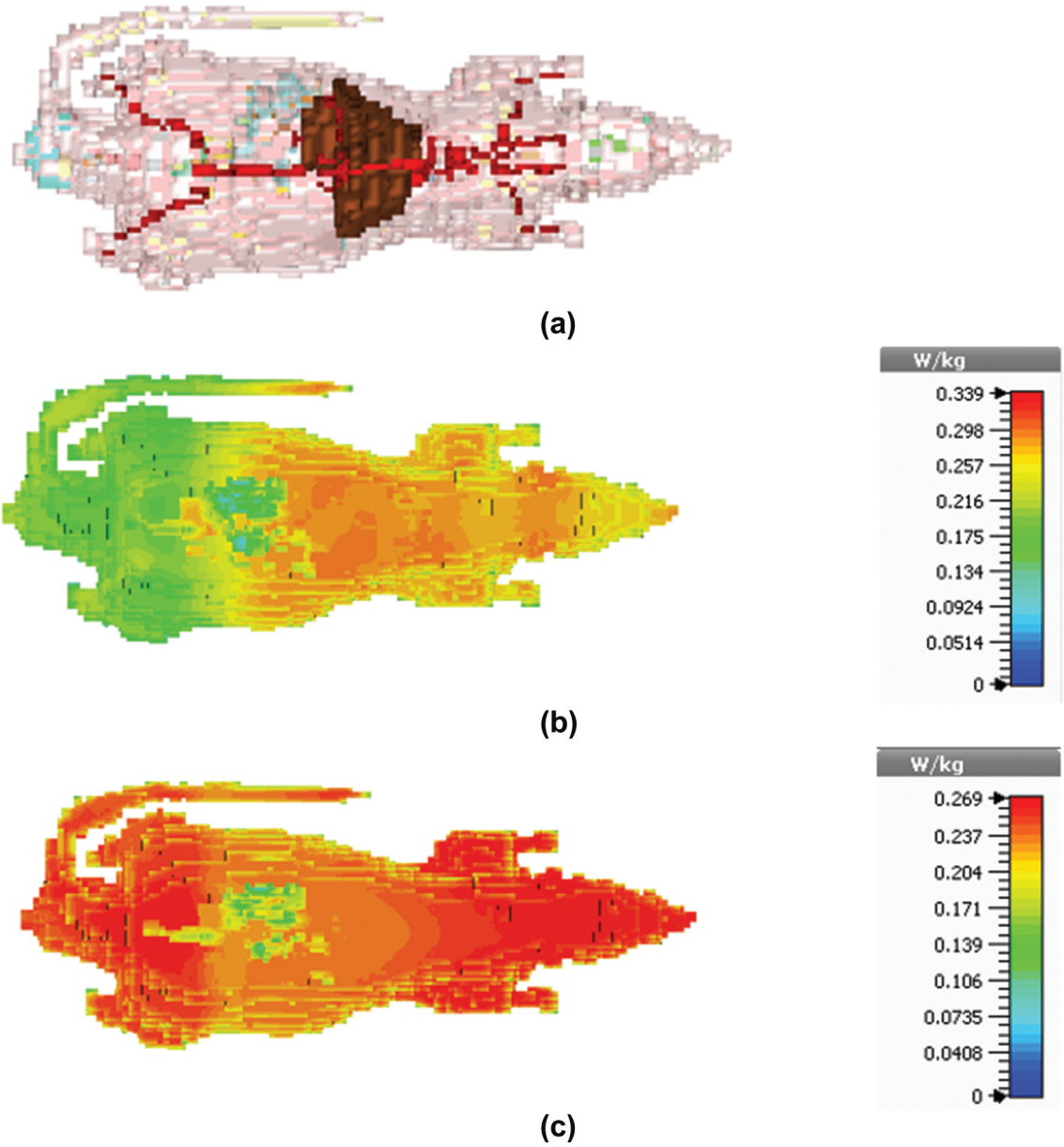


Figure 2. SAR model and distributions, a: voxel model with blood vessels and liver highlighted, b: 1 g average SAR distribution, C: 10 g average SAR distribution.

Results

Elisa test results

The results of the ROS assay showed that the ROS value in the RB group was statistically significantly increased compared to the C, B, and R groups, which is shown in Figure 3A ($p < .0001$, $p < .0001$, $p < .05$, respectively). No statistically significant difference in MDA measurements was found among the groups (Figure 3B).

According to the CAT results, as shown in Figure 4A, the level of CAT in the R and RB groups indicated a statistically significant decrease compared to the S ($p < .05$) and B groups ($p < .001$). There was no statistically significant difference between the control and bleomycin groups. Additionally, there was no statistically significant difference between the R and RB groups.

As shown in Figure 4B, the SOD inhibition rate in the R, B, and RB groups declined statistically significantly compared to the C group ($p < .05$) ($p < .001$) ($p < .0001$ respectively). The SOD inhibition rate in the RB group decreased statistically significantly compared to the R group ($p < .05$).

As can be seen in Figure 4C; the TAC level in the R and RB groups decreased statistically significantly compared to the C group ($p < .001$) ($p < .0001$ respectively). There is a statistically significant decrease in the TAC level of the RB group compared to the R and B groups ($p < .0001$) ($p < .0001$), respectively.

qRT-PCR results

p53 gene expression levels determined according to the qRT-PCR analyses are presented in Figure 5A. The p53 gene expression level of B group showed a statistically significant decrease compared to group C ($p < .001$). The p53 gene expression levels in the RB group were statistically significantly increased compared to the B group ($p < .05$). No statistically significant difference was determined in the expression levels between the C and R groups.

PARP-1 gene expression levels, determined according to the qRT-PCR analyses are presented in Figure 5B. There was a statistically significant decrease in expression level in groups B and RB compared to group C ($p < .0001$, $p < .001$, respectively). Additionally,

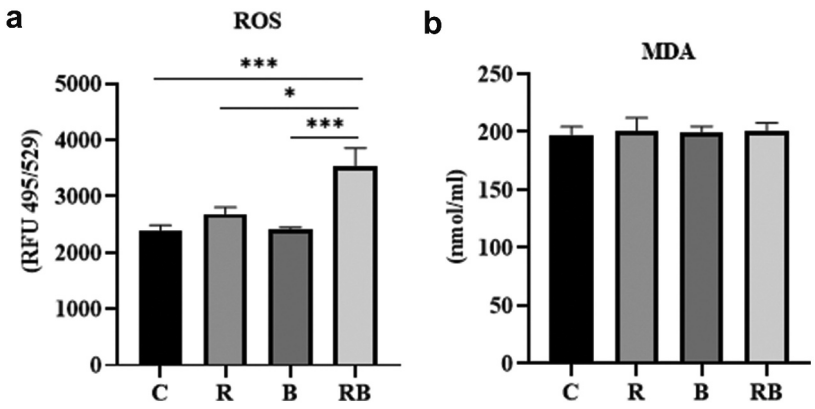


Figure 3. a: Reactive oxygen species (ROS), b: Malondialdehyde (MDA) levels in the plasma of mice. * $p < 0,05$, ** $p < 0,001$, *** $p < 0,0001$. All data are mean ± standard deviation.

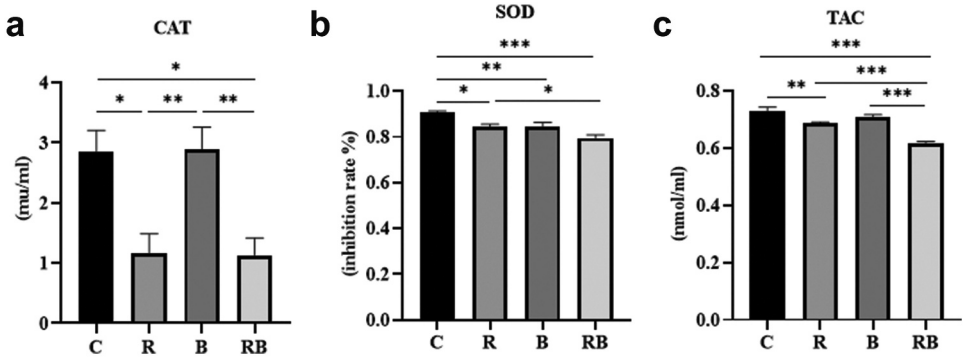


Figure 4. a: Catalase (CAT), b: Superoxide dismutase (SOD), and c: Total antioxidant capacity (TAC) levels in the plasma of mice. * $p < 0,05$, ** $p < 0,001$, *** $p < 0,0001$. All data are mean ± standard deviation.

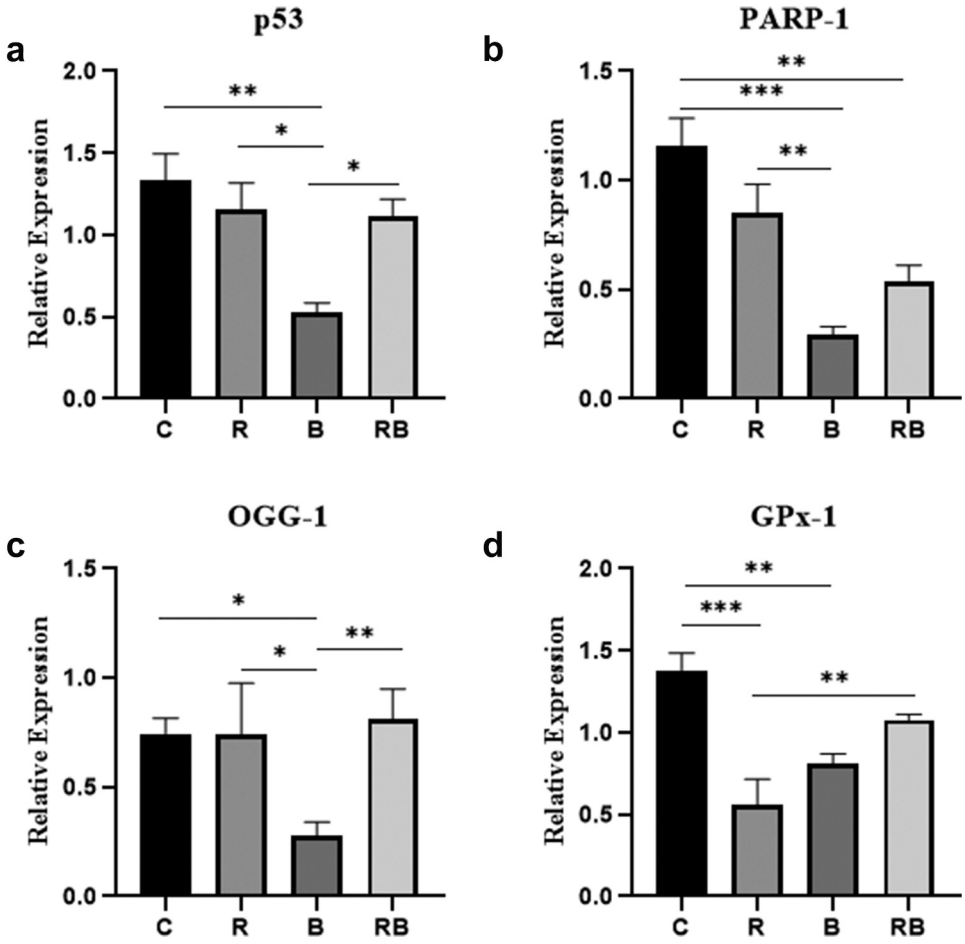


Figure 5. qRT-PCR based gene expression data for DNA repairing genes. a: p53 gene (tumor protein p53), b: PARP-1 gene (Poly (ADP-ribose) polymerase-1), c: OGG-1 gene (8-oxoguanine DNA glycosylase-1), d: GPx-1 gene (Glutathione Peroxidase 1). * p < 0,05, ** p < 0,001, *** p < 0,0001. All data are mean ± standard deviation.

there was no statistically significant difference in the expression levels of the R and RB groups.

OGG-1 gene expression levels are illustrated in (Figure 5C). The *OGG-1* gene expression level of B group showed a statistically significant decrease compared to group C ($p < .05$). The RB group's gene expression level increased statistically significantly compared to the B group ($p < .001$). But, not statistically significance difference was found neither between the C and R groups nor between the C and RB groups. Additionally, there was no statistically significant difference in the expression levels of the R and RB groups.

According to the *GPx-1* gene expression levels (Figure 5D), the gene expression levels in group B showed a statistically significant decrease compared to group C ($p < .001$). But no statistically significant difference was found between the RB and B groups. Gene expression levels in the R group showed a statistically significant decrease compared to the C and RB groups ($p < .0001$, $p < .001$, respectively).

TUNEL results

When the numbers of TUNEL positive cells in the liver tissues of the experimental groups were analyzed, no statistically significant difference was determined between the C, R and RB groups. The number of TUNEL positive cells in the B group increased statistically significantly compared to the C group and the R group ($p < .0001$ and $p < .0001$, respectively). There was no statistically significant difference between the R group and the RB group. The number of TUNEL positive cells in the RB group was statistically significantly lower than the B group ($p = .004$) (Figure 6E).

Discussion

RF exposure may trigger a cellular mechanism to protect cells from the effects of bleomycin. In previous studies, human blood lymphocytes were exposed to high doses of chemotherapeutic drugs or X-rays after RF exposure.

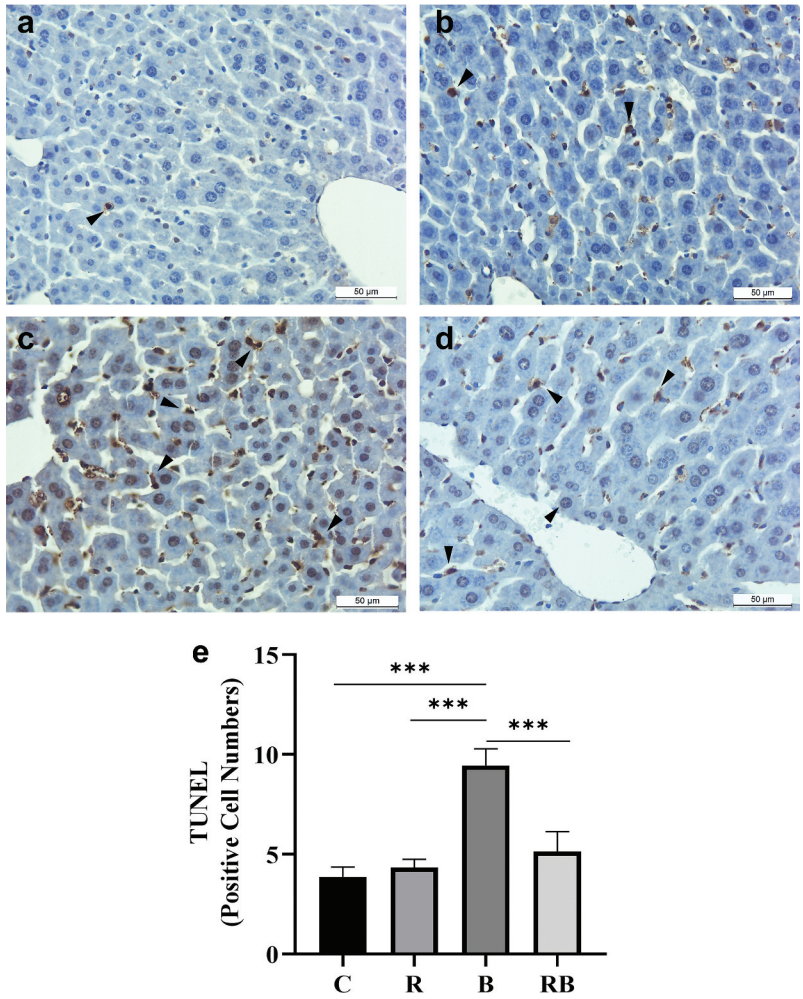


Figure 6. TUNEL-positive cells (arrowhead) in experimental groups. a: C; b: R; c: B; d: RB; e: TUNEL positive cell number * $p < 0,05$, ** $p < 0,001$, *** $p < 0,0001$. All data are mean ± standard deviation.

The group receiving RF exposure activated important defense mechanisms against genetic damage (Sannino et al. 2009, 2014; Zeni et al. 2012). The pre-RF field application has made animals resistant to the lethal level of ionized radiation and also to bacteria (Haghani et al. 2013; Mortazavi et al. 2012), but the mechanism of RF-induced AR has not yet been fully explained. In addition, we investigated the relationship between free radicals and the antioxidant mechanism of the AR process. ROS, CAT, MDA, SOD, and TAC protocols were used to determine the oxidative effects and free radical mechanisms assessed in the plasma.

The liver is a vital organ in mammals and plays key roles in detoxification, cholesterol production, glycogen storage, plasma protein synthesis, bile production and hormone production, etc (Debbi et al. 2017). Various reports have shown that low levels of electromagnetic fields can alter the liver function (Alkis et al. 2021; Ozgur et al. 2014; Sharma, Shrivastava, & Shukla, 2021). For these reasons, liver tissue was selected as the target organ. The cellular reaction to radiation and chemical therapeutic agents is a very complex process involving a cascade of concerted genes whose products may play a role in a wide range of activities, such as DNA repair, regulating the cell cycle, signal transduction, apoptosis, and oncogenesis (Sakamoto-Hojo et al. 2003).

The *p53* gene also plays an important role in the repair of radiation-induced DNA double-stranded breaks (Sasaki et al. 2002). The *p53* gene is activated in response to many stress stimuli, including the activation of oncogenes and DNA damage (Aubrey et al. 2018). Sasaki et al. (2002) reported that *p53* tumor suppressor protein played a key role in the adaptive response generated by ionizing radiation (Sasaki et al. 2002). In this study, the role of the *p53* gene in the RF-EMF-induced adaptive response has been shown in-vivo for the first time.

In the group exposed to BLM, the p53 gene expression level was statistically significantly decreased compared to the C group. This decrease was reversed with

the co-treatment of RF + BLM. RF restored the BLM-induced decline of p53 gene expression. This could mean that RF pre-exposure maintains the cell's ability to repair oxidatively damaged DNA intact.

The *PARP-1* gene plays a multifaceted role in the cellular response to DNA damage, with many pathways in the repair of DNA damage and in the protection of the genome (Pascal 2018; Ray Chaudhuri and Nussenzweig 2017). According to the results of our study, the *PARP-1* gene expression level in the R group increased statistically significantly compared to the B groups. After RF exposure, the expression level of the *PARP-1* gene involved in the repair mechanism increased and actively contributed to the formation of AR. In our study, however, there was no increase in the expression level of the *PARP-1* gene in the RB group.

PARP-1 and *p53* might play important roles in AR induced by low dose radiation (LDR) (Cheng et al. 2010). Similarly, these genes may also play an important role in RF-induced AR.

OGG-1 is a critical DNA repair enzyme (Sampath et al. 2012). Falone et al. (2018) reported that pre-RF exposure restored toxic agent induced down-regulation of *OGG-1* and *Gpx-1* (Falone et al. 2018). *OGG-1* gene expression increased in response to the oxidative stress induced by the increase of the ROS (Hao et al. 2018).

Furthermore, RF reversed the BLM-induced decrease in OGG-1 gene expression, suggesting that RF pre-exposure retains the cell's ability to repair oxidatively damaged DNA. Also, the level of OGG-1 gene expression, which was decreased with BLM, returned to the level of control with RF + BLM. In our study, the expression level of *OGG-1* gene increased with the increase of ROS in the RB group.

Falone et al. (2018) found that when menadione (MD) was applied to GPx-1 gene expression analysis revealed that the GPx-1 transcript was drastically reduced. RF pre-exposure of MD-treated cells restored the amount of GPx-1 to that observed in sham-exposed controls (Falone et al. 2018). In our study, gene expression of GPx-1 was significantly reduced by BLM and RF exposure. No statistical difference was observed between the RF pre-exposure of BLM-treated group and the control. Low GPx activity is especially fatal in situations of oxidative stress, such as therapy with anti-neoplastic drugs, and inhibition of GPx has been shown to be sufficient to change cell viability (Remacle et al. 1992; Vibet et al. 2008). The decrease in GPx-1 expression levels may have been due to BLM, an anti-neoplastic agent, and oxidative stress caused by ROS. RF pre-exposure of BLM-treated may have reversed this adverse effect and increased cell survival.

According to studies in the literature, minimal damage might trigger the activation of genes that play a role in DNA repair systems (Boreham and Mitchel 1991; Matsumoto et al. 2004; Wolff 1998). In our study, minimal stress caused by ROS may have induced the activation of genes involved in DNA repair systems.

We think that the application of RF prompts the production of free radicals/ROS in living cells. Unquestionably, free radicals can cause harmful effects due to the oxidation of organic macromolecules. In this way, even unpretentious exposures to RF of scarcely noticeable amounts of free radicals can have their important biological implications (Yakymenko et al. 2016). Since both excessive oxidative and anti-oxidative stress are harmful, it is important to keep the balance between ROS and antioxidants at an optimal level (Poljsak et al. 2013). Oxidative stress is a metabolic state characterized by an imbalance between ROS and antioxidative defenses. According to Dasdag and Akdag (2016), mobile phones and similar equipment can be considered as a factor causing oxidative stress (Dasdag and Akdag 2016). ROS plays an important role in generating the minimum level of damage required to trigger the induction of the adaptive response. In the R and B groups, there was no increase in the amount of ROS, while in the RB group, the amount of ROS increased statistically significantly. According to results of our study, ROS can play an important role in the stimulation of adaptive response by creating a minimum damage level. Nonetheless, one could hypothesize that helpful impacts of non-ionized radiation could be clarified by the activity of free radicals instigated at levels below the harming density. The potentially positive effects of external environmental influences can be viewed with suspicion until the long-term consequences are properly measured.

The adverse health effects of low-intensity RF fields give us a clear warning with direct oxidative damage or impaired cellular signaling (Yakymenko et al. 2016). In-vitro and in-vivo studies have shown that MDA, which is a marker of lipid damage, does not change in exposure to RF fields (Ferreira et al. 2006; Marjanovic Cermak et al. 2017). In our study, the MDA levels did not statistically significantly change among the groups. SOD and CAT are important antioxidant enzymes of the defense mechanism, converting active oxygen molecules into non-toxic compounds (Marjanovic Cermak et al. 2017). In the study by Zong et al. (2015), in which plasma SOD levels were measured in the liver and lung tissues of adult mice, they reported that the SOD level decreased in all tissues with BLM injection, and the SOD

level in the liver tissue increased only in the pre-RF-EMF group (Zong et al. 2015).

Oxidative stress is the result of increased free radical formation or reduced physiological activity of antioxidant defense against free radicals (Poljsak et al. 2013). Additionally, the ROS levels increased in the groups exposed to RF-EMF with the decrease of SOD, CAT, and TAC amount. Mitochondria are the essential source and one of the points of RF-induced ROS and abnormal mitochondria increase ROS levels in the cell (Sun et al. 2017). The electron transport chain is directly targeted by RF-EMF, resulting in mitochondrial dysfunction and excessive generation of ROS. EMF may alter mitochondrial proton motive force, causing the equilibrium between ROS generation and antioxidant enzymes to be disrupted. Extreme and long-term oxidative stress may have a role in mitochondrial dysfunction, which can lead to many different of health issues in humans (Santini et al. 2018).

Activating *p53* gene by stress can cause a variety of consequences including aging, differentiation, apoptosis, and cell cycle arrest. Under certain conditions, the *p53* gene also contributes to the repair of the genetic damage, allowing the rehabilitated cell to be released back into the proliferation pool (Vousden and Lu 2002). It has been shown that the low dose of ionizing radiation reduced apoptosis with the adaptive response (Feinendegen et al. 2004; Gandhi 2018; Wang et al. 2015).

The relationship between adaptive response caused by non-ionizing radiation and apoptosis has not been investigated at all. According to our results, cell death in the B group increased, while decreasing statistically significantly in the RB group. The RB group treated with BLM, may have provided protection against apoptosis after RF exposure by the increase in the expression of genes involved in the DNA repair mechanism. The increase in *p53*, GPx-1 and *OGG-1* genes expressions in the adaptive response group may have played an active role in the reduction of cell death.

In our experiments, RF exposure didn't uncover a statistically critical impact looking into mice, except for GPx-1. In any case, when RF- exposed mice were treated with BLM, we noticed an adaptive response that returned the BLM impact for several the endpoints evaluated. Moreover, the co-treatment with RF + BLM restored the effects of BLM on both p53 and OGG-1 transcript levels. The combined treatment also increased the gene expression level of ROS, leading to a possible improvement of the cellular response to reduced genotoxic damage.

If ROS, which increases with oxidative stress, is not decreased by antioxidant systems, it causes redox imbalance. This imbalance activates redox sensitive signaling pathways, which leads to responses including cellular responses, various protein synthesis (Miura and Endo 2010), increased cell proliferation (Murrell et al. 1990), and gene expression (Schreck et al. 1991). When looking at the balance between ROS and antioxidants in our study; failure to increase antioxidant levels in the RB group may not reduce the ROS level. Thus, it may have allowed different defense mechanisms to come into play. ROS produced by radiation can directly promote toxicity to DNA by causing changes in the structure and integrity of DNA. This can also eventually prompt *p53* signaling followed by cell cycle arrest and apoptosis. However, ROS can additionally alter the activity of DNA to restore mechanisms (Sisakht et al. 2020).

Looking at the molecular mechanisms involved in RF-induced AR, RF-EMF given at adaptive doses can cause slight stress in animals, which in turn can induce an AR. It can activate signal transduction pathways that lead to the activation of cell defense mechanisms by signaling pathways. Activated cell defense mechanisms enable the cell to later resist the damages caused by exposure to a challenging dose of a genotoxic agent. In our study, RF pre-exposure significantly reduced the damage caused by genotoxic agents with different mechanisms of action. Our recent findings are comparable with those described in the literature by researchers. We showed that apoptosis decreases with adaptive response by the TUNEL method. However, caspases and pathways should be examined in order to better understand the apoptosis mechanism of the adaptive response. This study, which was carried out only on healthy animals, is one of our recommendations to evaluate the effects of adaptive response on cancer and tumor tissue with cancer model mice. To achieve reliable results regarding the RF-induced AR molecular mechanisms, further studies are required.

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